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Morphology of Deoxyribonucleic Acid Extracted from Cores of Vaccinia Virus

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It has become apparent during the past few years that viral genomes may exist, at some stage of their life cycle, as an endless structure that can for convenience be termed a circle. The deoxyribonucleic acid (DNA) of small viruses such as polyoma appears to exist in this form inside the virion, since it can be extracted as rings a few microns in length (R. Weil and J. Vinograd, Proc. Natl. Acad. Sci. U.S. **50**:730, 1963); that from larger viruses, although perhaps existing in the virion as a two-ended macromolecule, may assume a circular form when released, as a result of the cohesion of opposite ends of the molecule [e.g., λ bacteriophage (A. D. Hershey, E. Burgi, and L. Ingraham, Proc. Natl. Acad. Sci. U.S. **49**:748, 1963)]. In the case of poxviruses, although high molecular weight DNA has been extracted by use of detergents both from poxvirions (W. K. Joklik, J. Mol. Biol. **5**:265, 1962) and from inclusion bodies (J. M. Hyde, C. C. Randall, and L. G. Gafford, Proc. Intern. Congr. Electron Microscopy, 6th, Kyoto, p. 193, 1966), no circular forms have so far been observed.

It has been shown recently that the outer layers of vaccinia virions can be removed selectively under mild conditions to yield a preparation that consists entirely of "cores" (K. B. Easterbrook, Ultrastruct. Res. **14**:484, 1966). Since these cores can be very easily ruptured and dissolved, they would seem to offer advantages over complete virions as starting material for the extraction of unbroken genomes. This note describes results obtained in an investigation into the morphology of DNA isolated from vaccinia viral cores by further detergent treatment.

The following procedure was followed. Vaccinia virus [Mill Hill strain (F. Fenner, Virology **5**:502, 1958)] was grown on the chorioallantoic membrane and was purified according to the methods of Joklik (W. K. Joklik, Virology **18**:9, 1962) to give a preparation which had an efficiency of plating of 1:10 (chick fibroblast assay). This was then treated successively, as previously described (K. B. Easterbrook, Ultrastruct. Res. **14**:484, 1966) with a nonionic detergent (Nonidet P40) and mercaptoethanol to release the cores. The cores were separated by centrifugation ($10^4 \times g$ for 30 min) and resuspended in tris(hydroxymethyl)aminomethane buffer (0.001 M, *p*H 8) at a concentration of about 10^{11} particles per ml. One volume of core suspension was mixed with two volumes of ammonium acetate (1 M) and one volume of sodium dodecyl sulfate (0.4%). After 15 min, a further volume of cytochrome *c* (0.5%) was added, and about 0.2 ml of the mixture was immediately spread by the Kleinschmidt method (A. K. Kleinschmidt and R. K. Zahn, Z. Naturforsch. **14b**:770, 1959) onto a hypophase of ammonium acetate (0.15 M). The cytochrome *c* film was compressed, and areas of the film were picked up by touching it with parlodion-coated electron microscope grids. The DNA was stained on the grids by immersion in an alcoholic solution of

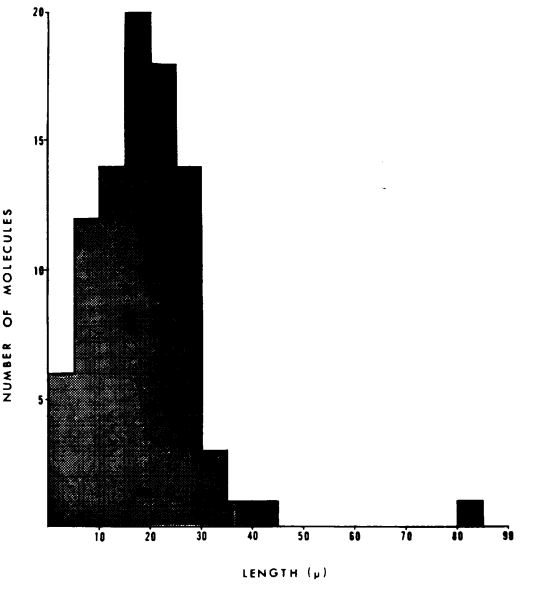

FIG. 1. *Distribution of lengths of molecules observed.*

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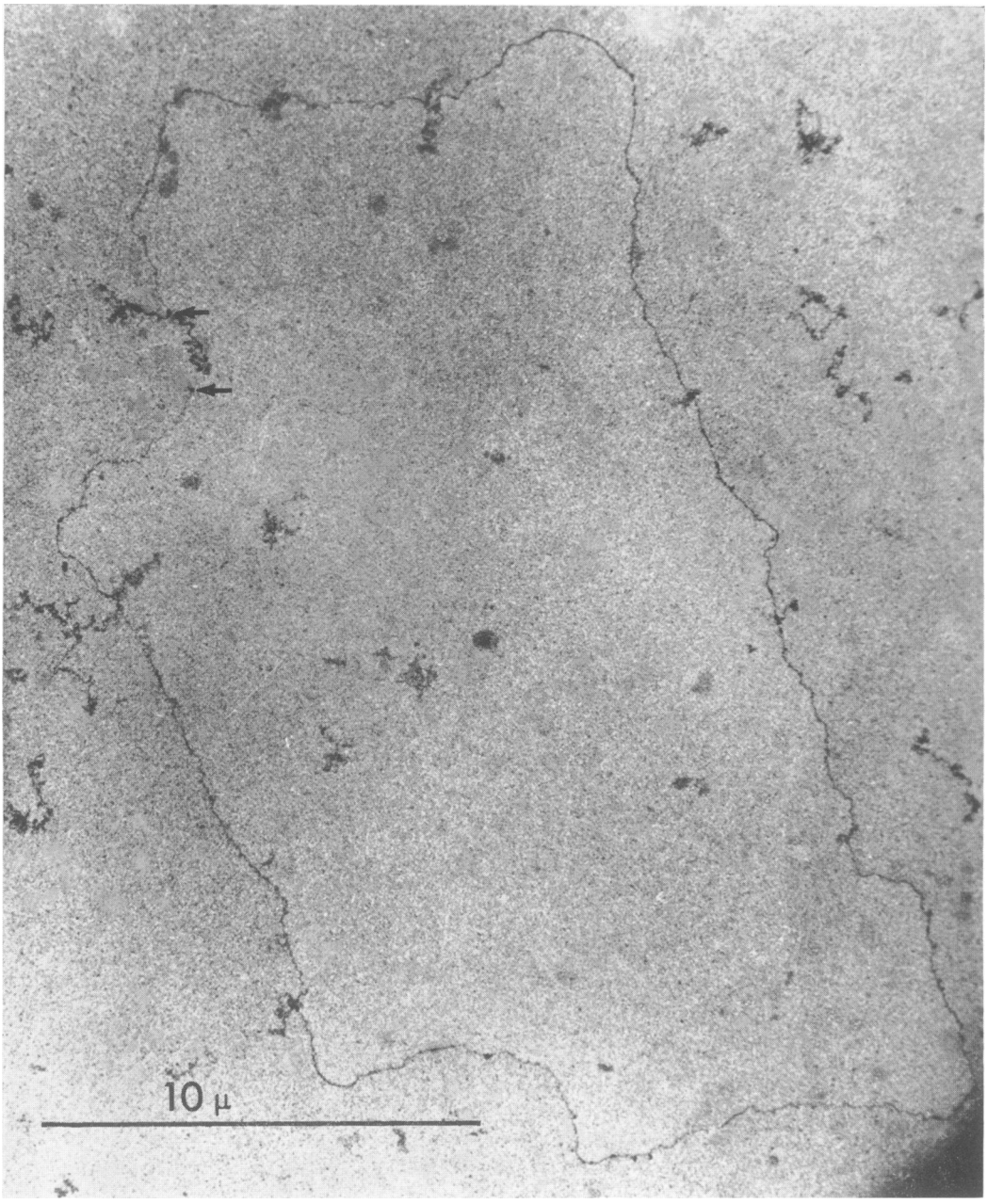

FIG. 2. *Morphology of the longest DNA molecule. The length, measured between the marks, is 83 μ.*

uranyl acetate (10^{-3} M in 95% ethyl alcohol) for 15 sec, followed by brief washing in absolute ethyl alcohol and isopentane. After air-drying, the grids were examined in an electron microscope (Phillips EM 200, 40 kv, 10-μ objective aperture, calibrated with a diffraction grating replica), and the stained molecules were photographed, usually at a magnification of 5,000 times. Measurements were made of the lengths of molecules with a map measurer after projecting the negatives on a screen and tracing over the magnified images with a pencil.

The distribution of lengths of the DNA molecules observed on a single grid is shown in Fig. 1.

It can be seen that 19 of the total of 90 measured had lengths greater than 25 μ, and one molecule, the integrity of which was checked at higher magnification, had a minimal measured length of 83 μ. The morphology of this latter molecule is shown in Fig. 2.

It can be calculated from estimates of particle weight and the DNA composition of vaccinia virus (*see* W. K. Joklik, J. Mol. Biol. **5**:265, 1962) that one particle contains on an average about 160 million daltons of DNA. The DNA of vaccinia is double-stranded (W. K. Joklik, J. Mol. Biol. **5**:265, 1962), and if it is assumed that it is in the B form with a weight-to-length ratio of 2 million daltons per micron (R. Langridge, H. R. Wilson, C. W. Hooper, and M. H. F. Wilkins, J. Mol. Biol. **2**:19, 1960), then this would correspond to an overall length of about 80 μ per virion. The close agreement between the calculated length and the maximum observed in the present investigation would suggest that the genome may exist in virions as a single molecule. If somewhat broad limits are allowed for asymmetrical breakage, and a single molecule of length 83 μ is the usual state of the genome, the sample described here would consist of one whole, five halves, and a number of molecules which have sustained on an average two successive breaks.

The shorter lengths of DNA observed to predominate in the sample studied could be due either to the existence of partial genomes in the virion or to subsequent experimentally induced breakage. The maturation of vaccinia virus is a somewhat random process, in that a developing protein shell encloses a volume of the replicating pool without prior condensation of the DNA. Only a few virions might, under these conditions, be expected to contain a complete molecule, and the low efficiency of plating of this virus group could be a reflection of this. On the other hand, the analysis of cowpox DNA by sedimentation and chromatography (W. K. Joklik, J. Mol. Biol. **5**:265, 1962) revealed that the molecules are apparently particularly sensitive to breakage as they are released from the constraint of the virion. Whatever the reason for small molecules, it has, nonetheless, been shown here that a single molecule of length equivalent to the calculated maximal molecular weight can be extracted from vaccinia virions.

It seems improbable that a two-ended molecule 83 μ long could assume fortuitously the configuration observed (Fig. 2) with the ends in such close apposition. It is tempting to suggest that the molecule is, in fact, derived from a previously endless molecule either by a single break, occurring perhaps on the grid during drying, or by separation of paired "sticky ends" such as exist in λ bacteriophage.

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